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# New and efficient microextraction/solid-phase extraction method for the gas chromatographic analysis of wine volatiles

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**Abstract**

A new method for analysing wine volatiles has been developed. The main features of the proposed method are (1) the minimal use of solvents to reduce overall analysis time and (2) the use of a silica solid-phase extraction cartridge to selectively separate the wine volatiles into two fractions prior to quantitative measurement by gas chromatography.

In this method a wine sample is first adjusted to 13% alcohol (v/v). An alcoholic fraction containing the majority of the volatile compounds is then obtained by salting-out the wine. Wine volatiles present in this fraction are micro-extracted by a liquid–liquid partition with 1,1,2-trichlorotrifluoroethane (freon 113). This freon extract is then applied onto a silica solid-phase extraction cartridge to selectively obtain two fractions that are directly injected into the gas chromatograph. Repeatability of the method is better than 5% (as R.S.D.) for more than 50 wine volatiles. Linearity was studied for all 50 compounds, and was satisfactory in most cases. Recovery of volatiles was checked by the analysis of spiked samples, and was also satisfactory. Detection limits with flame ionisation detection (FID) are below 1 μg/l for the best extracted compounds.

*Keywords:* Wine; Extraction methods; Solid-phase extraction; Volatile organic compounds

## 1. Introduction

The quantitative analysis of the volatile compounds present in food and drink is extremely demanding due to (1) the complex chemical composition of the volatile fraction and (2) the fact that individual volatile compounds can be present in a wide range of concentrations (from 1 ng/l to several g/l). These factors are particularly evident when wine volatiles are to be determined. For example, more than 99% of a wine flavour extract is composed of fusel alcohols, fatty acids and some fermentation esters, whereas the remaining 1% of the extract (which contributes significantly to the bouquet of a wine), is composed of hundreds of compounds, which are present at concentrations about $10^6$–$10^8$ times lower [1,2] than the fusel alcohols. The ultimate goal of volatile analysis is to obtain chemical information about the compounds that play an outstanding role in wine flavour, and this role depends on the concentration/sensory detection threshold ratio of the compound. As many of these components have exceedingly low sensory detection thresholds, analysis at concentrations as low as 1

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ng/l may be desirable. Therefore, methods for wine volatile analyses must be able to successfully quantify compounds over a wide range of concentrations.

In order to address these issues, different analytical strategies have been proposed. The most sensitive methods are those of total volatile analysis by means of continuous liquid–liquid extractions using solvents with low affinity to ethanol to extract wine volatiles [3–6]. These extractions yield quantitative recoveries of a wide range of compounds if the extraction time is long enough. However, these extracts are relatively crude, and some wine volatiles present at low concentrations, e.g. β-damascenone, can not be chromatographically resolved from those present at higher concentrations e.g. the fusel alcohols. A cleaner strategy is dynamic headspace analysis. This has been proposed under different forms: by the use of a trap containing an adsorbent [7], or by the ingenious combination of the purging system and a continuous liquid–liquid extraction with freon 11[1] [8]. However the sensitivity of these techniques is severely restricted by the volatility of the compounds to be analyzed, and compounds heavier than β-phenetyl alcohol can hardly be detected (see for instance [9]). Distillation has also been proposed [10] following the system developed by Forss et al. [11], but this is a rather complicated design, not suitable for routine analysis. The simultaneous distillation–extraction apparatus of Nickerson and Lickens [12], as modified by Shultz et al. [13] was also used for wine volatile analysis [14] and, although good results were obtained, we have not found additional references.

From a practical point of view, single-batch extractions can provide good analytical data for a limited number of compounds. Some of these techniques are often used for ester determination [15]. More recently, a method based on micro-extraction was proposed. This method makes it possible to quantitate twenty six volatile compounds [16], to obtain reproducible signals for near 50 compounds with detection limits in the low ppb range, and to avoid the tedious and not always quantitative solvent evaporation step [17]. These techniques are very convenient if the extraction processes are adequately controlled and calibrated, and demonstrate that it is not necessary to perform a large-volume extraction or a continuous liquid–liquid extraction to obtain good analytical results.

Notwithstanding the quality and concentration of the extracts, a better sensitivity can only be achieved by improving selectivity, i.e. if peak overlapping is avoided. This improvement can be achieved via selective detection with MS, or by introducing a fractionation step. It is the latter possibility that is explored in this paper.

Many different solutions to avoid major peaks overlapping minor peaks in the gas chromatographic separation of wine volatiles have been suggested. (1) The flavour extract is often partitioned with basic solutions to remove fatty acids [18]; (2) The extract is washed with 1,2-propylglycol to remove alcohols [3,18]; (3) The extract is separated with an open column containing silica gel to obtain two or three fractions [6]; (4) The extract is distilled and several fractions are collected [18]; (5) The extract is fractionated by HPLC [19]; (6) A dual-column gas chromatograph is used [20]. Options 1 and 4 do not solve the problem completely, because the improvement achieved is not good enough. Besides, option 2 removes some components of interest; option 3 requires the use of high amounts of solvent and has poor reproducibility; options 5 and 6 require additional instrumentation, and option 6 is only useful when the number of compounds to be determined is limited.

In this paper we have studied the combination of micro-extractive techniques [16,23] to reduce solvent consumption and hence eliminate the need of a concentration step. In order to separate the wine volatile extract into useful fractions, a sample fractionation technique based on pre-packed silica cartridges was developed. This fractionation step is simple, fast and reproducible, and requires only a few millilitres of solvent per sample. The need for further sample pre-concentration was avoided by

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[1] Large-scale production and use of CFCs must come to an end this year (1995) in accordance with the "Montreal Protocol" (see Ref. [24]). Title VI of the Clean Air Act permits limited continued production of CFCs after 1995 for specified essential uses, but probably these solvents will not be commercially available. For more information see Ref. [25].

large-volume solvent injections into the gas chromatographic column.

## 2. Materials and equipment

### 2.1. Solvents

All solvents were HPLC grade. Diethyl ether, pentane and dichloromethane were purchased from Rathburn (UK). Freon 113 (1,1,2-trichlorotrifluoroethane) was supplied by Aldrich (Gillingham, UK). Ethanol was from Merck (Darmstadt, Germany). Water was purified through a MilliQ system (Millipore, Badford, MA, USA). Solvents did not require additional distillation.

### 2.2. Chemical standards

Most were purchased from Aldrich (Gillingham, UK), Chemservice (West Chester, PA, USA), and Polyscience (Niles, IL, USA) and, where available, were of analytical grade. The source and grade can be seen in Table 4.

### 2.3. Standard solutions

Individual calibration solutions: exact weights ($>0.03 \pm 0.0001$ g) of the chemical standard compounds were dissolved in absolute ethanol and made up to volume (10 ml).

Mixed standard solution: chemical standards were dissolved in ethanol at concentrations typically found in wine [2]. This solution was then diluted with water and/or alcohol (adjusting final alcohol content to 13% by volume) to prepare calibration graph(s) and to spike different wine samples.

Internal standard solution: ethyl pentanoate and ethyl undecanoate (apolar standards at 0.2 mg/ml), and 4-methyl-2-pentanol, 2-octanol and dodecanol (polar standards, 0.4 mg/ml) were dissolved in ethanol.

Saline solution: ammonium sulphate [$(NH_4)_2SO_4$, 95 g] was dissolved in water (200 ml).

### 2.4. Solid-phase extraction columns

Silica and diol cartridges: 500 mg from Varian (Harbor City, USA; Bond Elut) and Waters (MA, USA; Sep Pack).

### 2.5. Gas chromatography conditions

Sample fractions were analysed with a Carlo Erba Mega series gas chromatograph under the following conditions:

Analytical column (J&W, DB-WAX, 50 m×0.32 mm; 1.2 $\mu$m film thickness) preceded by a retention gap deactivated with methyl phenyl siloxane (15 m×0.53 mm). Hydrogen was used as the carrier gas (3 ml/min).

Injection conditions, cold on-column injection. Vial pressure, 2 kPa (helium); transfer time, 25 s (approximately 0.1 ml).

Temperature program: 40°C for 20 min, then raised to 200°C at 3°C/min.

### 2.6. Gas chromatography–mass spectrometry conditions

An HP 5890 Series II chromatograph fitted with a 5971A electron-impact detector was used. The detector was operated in the scan mode over 35 to 300 amu with a 0.4-s dwell time. Spectra were stored and processed with MS Chemstation Series II software, which contained the Wiley MS library.

Analytical column, same column as for GC–FID. Helium carrier at 50 kPa.

Injection conditions, splitless (1.5 min). Injected volume, 1 $\mu$l of a 1000-fold concentrated extract.

Temperature program: initial temperature 40°C, held for 5 min and then raised at 3°C/min to 220°C.

## 3. Method development

### 3.1. Wine pre-concentration (by salting-out)

The weights of ammonium sulphate and sodium dihydrogen phosphate added to samples of wine were varied until the optimum separation of ethanol from water was achieved. At the same time, the

alcohol content of wine samples was enriched with varying amounts of absolute ethanol, until the wine volatiles recovered by the salting-out procedure was maximised.

### 3.2. Solid-phase silica cartridge selection

Silica and diol cartridges (500 mg) from Waters and Varian were investigated. Retention of analytes was studied in both standard solutions and wine extracts. Elution solvents (pure pentane, dichloromethane and diethyl ether and the elutropic series pentane–dichloromethane and pentane–diethyl ether, 2, 5, 10, 25 and 50% in the polar solvent) were applied to the column. Silica cartridges from Varian were selected for further study, as they afforded the best potential for separating wine volatiles into suitable fractions prior to quantitation by gas chromatography.

### 3.3. Optimisation of solid-phase fractionation of wine volatiles

To avoid the possibility of sample contamination, Silica columns (500 mg, Varian Bondelut) were prewashed with diethyl ether (6 ml) and then dried under a stream of nitrogen. A freon 113 wine extract (see proposed method below) was applied to the pre-conditioned columns, and the elution characteristics of a series of solvents (pentane, dichloromethane, chloroform and diethyl ether) were investigated. Conditions leading to the maximum retention and subsequent separation of fusel alcohols from other wine components present in the freon 113 extract were chosen. Maximum analyte loading was studied by increasing the amounts of freon 113 wine extract applied to the column.

## 4. Proposed method

### 4.1. Extraction

Solution A: add internal standard solution (25 $\mu$l) to a volumetric flask (50 ml) and make up to volume with the wine sample. Shake to mix. Solution B: add the necessary amount of ethanol to a second volumetric flask (50 ml) in order to increase the ethanol content of the wine (used to prepare solution A) to 13% (v/v). Finally, add solution A to bring the second volumetric flask to volume.

To a dry separating funnel (250 ml), add sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$, 6.6 g), ammonium sulphate [$(NH_4)_2SO_4$, 27 g] and, finally, the contents of the solution B containing the wine normalised to 13% (v/v) ethanol. Shake until salt is completely solved (ca. 10 min). Once the phases have been separated, pipette an aliquot of the supernatant organic phase (2 ml) into a screw-capped centrifuge tube (15 ml). Finally, add saline solution (10 ml) and freon 113 (0.2 ml) to the centrifuge tube. Cap the tube and leave it shaking fast for approximately 1 h.

### 4.2. Clean-up

After shaking the sample for 1 h, centrifuge the tube at 1600 *g* for 5 min. Recover the freon extract with a 0.5-ml syringe and apply it slowly onto a previously cleaned (with 6 ml ether) silica cartridge. Elute the first fraction slowly with dichloromethane (4 ml) into a vial with a PTFE septum. Then elute a second fraction with diethyl ether (4 ml) into a separate vial.

### 4.3. Measurement of analyte concentration

Inject both fractions obtained from the silica cartridge onto the gas chromatograph under the conditions listed above. Calculate the relative response areas for each of the individual wine volatiles to the appropriate internal standards (ethyl pentanoate for the dichloromethane fraction and 2-octanol for the ether fraction). Interpolate the relative responses in the corresponding calibration graphs prepared from standards (see below).

### 4.4. Calibration graphs (prepared from synthetic wine solutions)

To prepare "synthetic wine solutions", add ethanol [until a final concentration of 13% (v/v)], tartaric acid (6 g/l) and known amounts of chemical standards of the wine volatiles to be studied to water. Adjust the combined solution to pH 3.2 with sodium hydroxide (1 *M*). The concentration of these chemi-

cal standards should be chosen so that the typical concentration range found in wines is covered [2]. For example, in this study, we prepared standards that covered a range of 2 orders of magnitude, with approximately 8 calibration points for each standard. Finally, analyse the synthetic wine solutions following the afore-mentioned method. Use these data to construct the calibration graphs.

## 5. Results and discussion

### 5.1. General

In this paper we have followed a strategy close to the classical approaches; organic solvents were used to extract the wine volatiles, and normal-phase chromatography was used to effect the separation. Whilst this particular choice has some limitations (e.g. the major compounds (e.g. fusel alcohols) are strongly retained in these systems, thereby the overall performance of the method is restricted by major compounds and not by minor solutes), there are plenty of potential advantages. In particular, the highly selective character of normal-phase packing ensures that good isolation of components of interest are achieved during the extraction and separation processes. Furthermore, the use of volatile solvents (such as ether) ensures that large-volume injections can be performed on the gas chromatograph without affecting the performance of the analytical separation of these wine volatiles.

### 5.2. Extraction

In general, when wine volatiles flavours are to be extracted, the best recoveries are obtained by using continuous liquid–liquid extraction techniques [5,6]. However, the major drawback of this approach is that large volumes of solvents are required. It has been previously demonstrated that micro-extraction of wine volatiles with freon 113 (0.2 ml) eliminates the need of solvent evaporation whilst maintaining good analytical characteristics [8]. Furthermore, the limit of detection of this approach can be improved by a pre-concentration step. In this case salt(s) are added to the wine sample to produce an alcohol-enriched liquid phase which contains the majority of the wine volatiles. This pre-concentration technique was previously applied to wine flavour analysis by Ribereau-Gayon et al. [21] and Bertrand [22]. In this paper we have combined both of these procedures to produce a new method for analysing wine volatiles: Firstly, the alcoholic content of the wine is adjusted to 13% (v/v). Salt is then added to an aliquot of the wine (50 ml) to produce an organic phase [of approximately 60% (v/v) ethanol], which is enriched in wine volatiles (ca. 2.7 ml). A portion of this organic phase is then diluted with saline solution (10 ml). This maximises the micro-extraction of wine volatiles from the organic phase with freon 113, whilst minimising the risk of emulsion formation which would affect solvent recovery.

### 5.3. Fractionation

Two types of solid-phase extraction cartridges (silica and diol, each 500 mg) from two manufacturers (Waters and Varian) were evaluated. The best results were obtained with silica cartridges from Varian. However, they could not be used directly owing to impurities that caused severe chromatographic interferences. Therefore a pre-cleaning step with diethyl ether was employed. Diethyl ether elutes any contaminants (such as plasticisers), and residual ether can be easily removed by drying with a stream of nitrogen. After this treatment, the gas chromatographic separation of reagent blanks was free from interferences, and the elution characteristics and maximum sample load of the solid-phase cartridges were improved. Three primary elution solvents were studied; dichloromethane, chloroform and diethyl ether. Dichloromethane and chloroform were also investigated after dilution with pentane [5, 20, 50 and 100% (v/v)]. Diethyl ether strongly eluted alcohols, phenols and acidic compounds from the cartridge, thus making it impossible to separate the less polar from the more polar compounds. Therefore, diethyl ether was chosen as the most appropriate solvent to elute the second "polar" fraction. Chloroform showed a poor selectivity and the elution of the less polar compounds (e.g. fatty acid esters) required several bed volumes. However, dichloromethane was extremely efficient at eluting these less polar compounds, whilst retaining the most polar volatiles on the column. Consequently, dichlorome-

thane was chosen as the most appropriate solvent to elute the first "non-polar" fraction. Combinations of dichloromethane and chloroform with pentane did not offer any extra selectivity and were not further explored.

Table 1 shows the elution pattern when the optimum conditions are employed. A number of basic elution patterns can be observed. Fatty acid ethyl esters, fusel alcohol acetates, and some aldehydes and ketones are eluted in the first few millilitres (0–2 ml) of the dichloromethane fraction, whereas volatile phenols are eluted in both dichloromethane fractions (0–2 and 2–4 ml). Most of the polar compounds are eluted in the first few millilitres (0–2 ml) of the ether fraction and, finally, fatty acids, are eluted incompletely in the second ether washing (2–4 ml). Therefore, a near perfect separation between the least and the most polar compounds can be achieved. In the final method the two dichloromethane fractions were combined to produce a single dichloromethane solution (4 ml). The two ether fractions were also combined. Fig. 1 and Fig. 2 show the chromatograms corresponding to the combined dichloromethane and ether fractions for a typical red wine.

The maximum loading of silica cartridges with wine volatiles is a critical parameter, owing to the reasons stated before. For example, a slight excess in fusel alcohols will result in some of the volatile compounds which are typically eluted in the second (ether) fraction, to be displaced into the first (dichloromethane) fraction. Since the amount of fusel alcohols present in a wine is variable, this effect could significantly reduce the ability of the method to quantify these compounds accurately. During our preliminary investigations, this effect was most noticeable when more than 60 ml of wine were used. However, the possibility that this problem will occur

Table 1
Elution properties of the silica solid-phase extraction system

| Compound | Recovery (%) (dichloromethane, 0–2 ml) | Recovery (%) (dichloromethane, 2–4 ml) | Recovery (%) (ethyl ether, 0–2 ml) | Recovery (%) (ethyl ether, 2–4 ml) |
|---|---|---|---|---|
| Isobutyl acetate | 97 | <3 | – | – |
| Isoamyl acetate | 94 | 5 | – | – |
| Amyl acetate | 100 | <1 | – | – |
| Hexyl acetate | 98 | <3 | – | – |
| Benzyl acetate | 99 | tr | – | – |
| Phenylethyl acetate | 96 | 4 | – | – |
| Ethyl hexanoate | 97 | <3 | – | – |
| Ethyl heptanoate | 99 | tr | – | – |
| Ethyl octanoate | 98 | <3 | – | – |
| Ethyl decanoate | 98 | <2 | – | – |
| 2-Heptanone | 100 | – | | |
| Hexanal | 100 | – | | |
| Benzaldehyde | 98 | <2 | – | |
| Acetophenone | 96 | 4 | – | |
| Guaiacol | – | <2 | 97 | – |
| *m*-Cresol | 64 | 36 | tr | |
| *o*-Cresol | 44 | 54 | 7 | – |
| Eugenol | tr | 6 | 93 | – |
| 4-Ethylphenol[a] | tr | 71 | 27 | – |
| Ethyl cinnamate[a] | 52 | 47 | – | |
| *β*-Ionone | – | <2 | 97 | – |
| Linalool | – | – | 100 | tr |
| Decanol | – | – | 100 | – |
| Octanoic acid | – | – | – | 10 |

tr=traces. [a] Compounds whose recovery is not complete in one of the fractions.

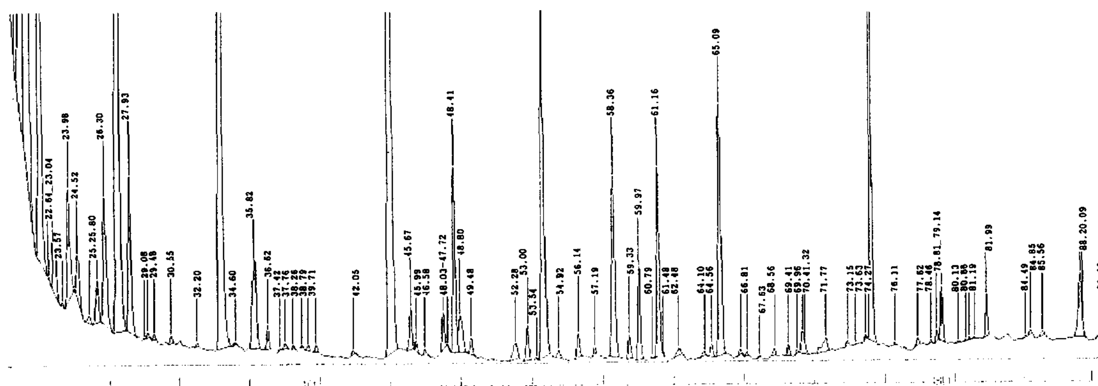

Fig. 1. Chromatogram of the apolar fraction (dichloromethane fraction) of a Spanish red wine. Peak information can be found in Table 2. Retention time in min.

in the proposed method is negligible, as the extract separated on the silica column is produced from a relatively small volume of wine (37 ml).

### *5.4. Analytical characteristics of the proposed method*

Table 2 shows the analytical performance of the proposed method. Compounds in the table were identified (1) by comparing their retention times and mass spectra with those of the pure chemical standard (calibrated compounds), or (2) only through their retention indexes and mass spectrometric data (non calibrated compounds). If the compound was not present in the calibration graphs used in the study, only its relative area was used. For each of the fractions (dichloromethane and diethyl ether), the mean concentration for the individual volatile compounds in a red wine sample is listed. The repeatability (in terms of % R.S.D.) associated with both the chromatographic measurements and the overall method are also presented. In order to determine the repeatability of the chromatographic procedure, the same wine extract was injected six

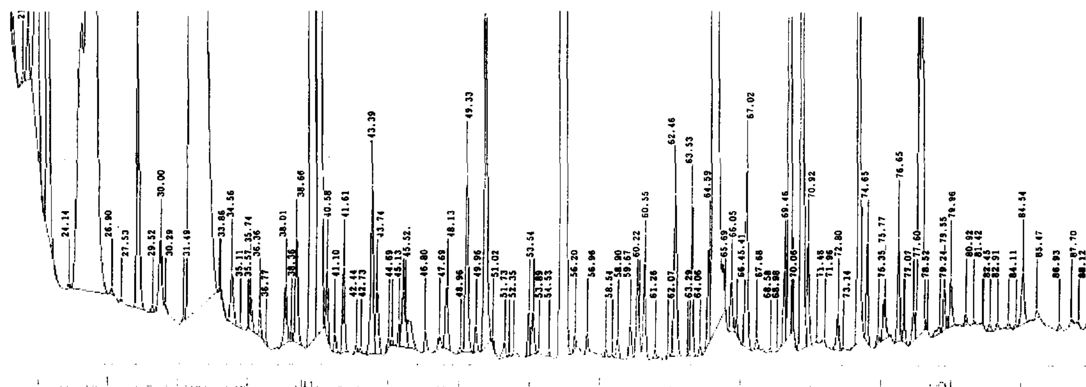

Fig. 2. Chromatogram of the polar fraction (diethyl ether fraction) of a Spanish red wine. Peak information can be found in Table 2. Retention time in min.

Table 2
Analytical performance of the proposed method: analysis of a single red wine (in-house reference material)

| Retention time (min) | Compound | Conc. (μg/l) | Chromatography R.S.D. (%) (n=6) | Overall method | |
|---|---|---|---|---|---|
| | | | | Repeatability R.S.D. (%) (n=6) | Long-term performance R.S.D. (%) (n=12) |
| *Fraction A (dichloromethane)* | | | | | |
| 18.7 | Propyl acetate | n.c. | 0.6 | 1.6 | 1.9 |
| 19.9 | Ethyl propanoate | n.c. | 1.9 | 2.7 | 1.7 |
| 22.3 | Isobutyl acetate | 92 | 2.2 | 3.3 | 2.6 |
| 23.0 | Ethyl butyrate | n.c. | 0.5 | 3.4 | 3.0 |
| 23.9 | Ethyl isovalerate[a] | n.c. | 0.8 | 2.2 | 3.4 |
| 24.5 | Hexanal | 42 | 0.8 | 2.7 | 2.8 |
| 25.3 | Unknown | n.c. | 4.6 | 7.3 | 8.4 |
| 25.8 | Unknown | n.c. | 2.1 | 3.9 | 3.8 |
| 26.3 | Ethyl benzene | n.c. | 3.2 | 11.3 | 14.2 |
| 27.1 | Isoamyl acetate | 628 | 1.2 | 3.0 | 2.9 |
| 27.9 | Ethyl pentanoate (I.S.) | | | | |
| 29.5 | Ethyl butenoate | n.c. | 8.5 | 9.2 | 8.4 |
| 30.6 | 2-Heptanone | 2 | 3.1 | 5.6 | 4.7 |
| 33.7 | Ethyl hexanoate | 556 | 1.1 | 3.1 | 3.3 |
| 35.8 | Hexyl acetate | 52 | 0.9 | 4.2 | 3.9 |
| 36.6 | *cis*-3-Hexenyl acetate[a] | n.c. | 1.3 | 2.9 | 3.1 |
| 39.7 | Ethyl 2-hexenoate | n.c. | 6.2 | 13.1 | 11.3 |
| 44.4 | Ethyl octanoate | 629 | 0.9 | 3.3 | 2.3 |
| 45.7 | Furfural | n.c. | 2.1 | 5.1 | 6.2 |
| 47.7 | *cis*-Vitispirane[a] | n.c. | 3.2 | 4.1 | 5.4 |
| 48.4 | Benzaldehyde | 126 | 1.2 | 3.0 | 2.9 |
| 53.0 | Ethyl furancarbonate | n.c. | 0.7 | 1.8 | 1.9 |
| 54.0 | Ethyl decanoate | 191 | 0.5 | 2.4 | 2.0 |
| 54.9 | Phenyl ethanone | 1 | 4.1 | 10.2 | 9.9 |
| 56.1 | Benzyl acetate | 12 | 2.5 | 3.0 | 3.1 |
| 58.4 | Ethyl undecanoate (I.S.) | | | | |
| 59.3 | TDN | n.c. | 8.7 | 16.1 | 11.2 |
| 60.0 | Ethylphenyl acetate | n.c. | 1.6 | 2.9 | 2.6 |
| 61.2 | Phenylethyl acetate | 46 | 1.5 | 2.0 | 1.7 |
| 62.5 | β-Damascenone | n.c. | 5.6 | 9.2 | 7.9 |
| 65.1 | Impurity | | | | |
| 68.6 | *m*-Cresol | 3 | 2.7 | 8.1 | 7.5 |
| 74.6 | 4-Ethylphenol | 145 | 1.9 | 11.9 | 12.3 |
| 79.1 | Unknown | | 1.5 | 2.3 | 3.3 |
| 82.0 | Unknown | | 3.2 | 15.8 | 12.5 |
| 88.1 | Vanillin | n.c. | 2.1 | 6.1 | 5.8 |

*(Continued on page 255)*

times in rapid succession. These data show that the R.S.D. for the gas chromatographic measurement is better than 5% for over 60 wine volatiles. To evaluate the repeatability of the whole procedure, the same wine sample was also analysed six times following the whole procedure. In this case, over 50 wine volatile compounds had an R.S.D. of 5% or less. Furthermore, for all other wine volatile compounds measured by this new method, the repeatability was typically between 5 and 10% R.S.D.

A long-term performance assessment of the new method data was obtained by analysing the same red wine sample (an "in house reference sample") for a further 12 times over more than 2 months. These

Table 2
Continued

| Retention time (min) | Compound | Conc. (μg/l) | Chromatography R.S.D. (%) (n=6) | Overall method | |
|---|---|---|---|---|---|
| | | | | Repeatability R.S.D. (%) (n=6) | Long-term performance R.S.D. (%) (n=12) |
| *Fraction B (diethyl ether)* | | | | | |
| 23.2 | Propanol | n.c. | 2.0 | 2.2 | 3.7 |
| 24.8 | Isobutanol | o.r. | | | |
| 28.8 | 1-Butanol | 204 | 0.3 | 1.0 | 0.5 |
| 32.2 | Isoamyl alcohol | o.r. | | | |
| 34.6 | 1-Pentanol | n.c. | 4.1 | 5.3 | 7.2 |
| 36.4 | Acetoine | 83 | 3.0 | 4.1 | 4.3 |
| 38.0 | 4-Methylpentanol | n.c. | 1.2 | 3.3 | 2.8 |
| 38.7 | 3-Methylpentanol | n.c. | 0.9 | 1.8 | 2.9 |
| 39.6 | Ethyl lactate | 21800 | 0.4 | 2.0 | 2.3 |
| 40.1 | Hexanol | 1550 | 1.4 | 2.0 | 1.5 |
| 40.6 | *cis*-2-Hexenol | n.c. | 0.9 | 1.2 | 1.5 |
| 41.1 | 3-Ethoxy-1-propanol | n.c. | 1.7 | 3.5 | 3.9 |
| 41.6 | *cis*-3-Hexenol | 117 | 0.7 | 0.8 | 1.5 |
| 42.7 | *trans*-2-Hexenol | 83 | 8.5 | 9.2 | 8.6 |
| 43.4 | 2-Octanol (I.S.) | | | | |
| 43.7 | Unknown hydroxy ester | | 0.7 | 0.8 | 0.6 |
| 45.5 | Heptanol | 24 | 7.7 | 8.9 | 10.2 |
| 46.8 | 2-Ethylhexanol | 14 | 1.2 | 1.3 | 1.1 |
| 49.3 | 3-OH-methyl butyrate | 117 | 0.7 | 1.0 | 0.8 |
| 50.0 | 1-Octanol | 19 | 1.4 | 2.0 | 3.0 |
| 50.5 | Diethyl malonate | 1283 | 1.3 | 1.7 | 1.6 |
| 51.0 | 5-Methyl furfural | n.c. | 6.6 | 7.6 | 7.2 |
| 53.3 | Unknown dioxolane | n.c. | 1.7 | 4.2 | 5.0 |
| 53.5 | Methyl ethyl succinate | n.c. | 2.1 | 3.1 | 4.4 |
| 55.3 | Diethyl succinate | o.r. | | | |
| 56.2 | α-Terpineol | 7 | 4.0 | 4.3 | 5.0 |
| 58.9 | 1-Decanol | 12 | 3.6 | 5.0 | 6.0 |
| 59.7 | Citronelol | 9 | 1.6 | 1.7 | 2.4 |
| 60.6 | Nerol | 12 | 1.7 | 2.3 | 2.3 |
| 62.5 | Guaiacol | 15 | 1.0 | 1.9 | 2.7 |
| 63.5 | Benzyl alcohol | 103 | 0.3 | 0.9 | 2.7 |
| 64.6 | Diisopropyl succinate[a] | n.c. | 0.3 | 0.6 | 0.8 |
| 64.7 | β-Phenylethanol | o.r. | | | |
| 66.1 | β-Ionone | 9 | 0.8 | 0.9 | 1.5 |
| 67.0 | 2-Octanol (I.S.) | | | | |
| 67.6 | Phenol | n.c. | 10.8 | 12.3 | 14.3 |
| 69.5 | γ-Nonalactone | 27 | 2.3 | 3.6 | 5.6 |
| 69.7 | Diethyl hydroxysuccinate | n.c. | 1.8 | 2.7 | 3.4 |
| 70.6 | Octanoic acid | n.c. | 2.9 | 4.2 | 5.9 |
| 74.0 | 3-Ethyl phenol+eugenol | n.c. | 0.6 | 2.1 | 2.5 |
| 74.7 | 4-Ethyl phenol | 28 | 1.0 | 3.2 | 3.7 |
| 75.8 | γ-Undecalactone[a] | n.c. | 2.2 | 3.0 | 3.4 |
| 76.7 | Unknown | n.c. | 2.3 | 5.2 | 5.7 |
| 77.1 | Unknown γ-lactone | n.c. | 1.4 | 4.8 | 3.6 |
| 78.0 | Decanoic acid+unknown | n.c. | 1.2 | 2.4 | 2.9 |
| 79.6 | Unknown | n.c. | 1.7 | 3.9 | 4.1 |
| 80.0 | Unknown | n.c. | 1.1 | 3.2 | 3.7 |

n.c.=Not calibrated; o.r.=Out of range; Retention times refer to Figs. 1 and 2.
[a] Tentative identification.

Table 3
Calibration graph data

| Compound | Intercept | Slope | r | Linear range (μg/l) | n | Fraction |
|---|---|---|---|---|---|---|
| *Esters* | | | | | | |
| Amyl acetate | 0.0033 | 17.40 | 0.9991 | 2.5–63 | 5 | A |
| Diethyl malonate | 0.175 | 2.02 | 0.9993 | 113–5000 | 6 | B |
| Diethyl succinate | 0.323 | 2.66 | 1.0000 | 500–50000 | 4 | B |
| Ethyl cinnamate | 0.019 | 12.30 | 0.9999 | 1.43–143 | 7 | A |
| Ethyl decanoate | 0.149 | 13.10 | 0.9994 | 88–9000 | 7 | A |
| Ethyl heptanoate | 0.0173 | 11.52 | 0.9988 | 1.9–191 | 7 | A |
| Ethyl hexanoate | 0.0399 | 12.50 | 0.9998 | 200–8000 | 6 | A |
| Ethyl lactate | −0.0037 | 0.04 | 0.9991 | 1000–50000 | 7 | B |
| Ethyl laurate | 0.0322 | 13.50 | 0.9991 | 4.5–480 | 7 | A |
| Ethyl octanoate | 0.0198 | 12.80 | 1.0000 | 120–10000 | 6 | A |
| 3-Hydroxybutyrate | −0.0061 | 3.27 | 0.9999 | 2–114 | 6 | B |
| Benzyl acetate | 0.00161 | 12.00 | 0.9998 | 1.73–43.2 | 5 | A |
| Hexyl acetate | 0.0685 | 10.60 | 0.9992 | 9–950 | 7 | A |
| Isoamyl acetate | 0.111 | 10.84 | 0.9995 | 240–10000 | 6 | A |
| Isobutyl acetate | 0.0144 | 19.30 | 0.9998 | 2.6–134 | 6 | A |
| Phenylethyl acetate | 0.0655 | 10.80 | 0.9991 | 2–135 | 7 | A |
| *Alcohols* | | | | | | |
| 2-Butoxyethanol | 0.065 | 6.33 | 0.9997 | 1.72–43 | 5 | A |
| 2-Ethylhexanol | −0.0016 | 7.47 | 1.0000 | 1.28–128 | 8 | B |
| 2-Heptanol | −0.0063 | 5.84 | 0.9999 | 1.3–130 | 7 | B |
| β-Phenylethanol | 0.056 | 0.57 | 0.9989 | 750–16000 | 5 | B |
| Benzyl alcohol | −0.0011 | 0.44 | 0.9920 | 8–200 | 5 | B |
| Butanol | −0.007 | 0.57 | 0.9991 | 11–584 | 6 | B |
| *cis*-3-Hexenol | −0.0123 | 1.58 | 0.9996 | 5–500 | 7 | B |
| Decanol | 0.00284 | 8.19 | 1.0000 | 0.81–40 | 7 | B |
| Heptanol | −0.0344 | 12.30 | 0.9998 | 0.8–80 | 7 | B |
| Isoamyl alcohol | −0.213 | 1.04 | 0.9998 | 500–10000 | 4 | B |
| Isobutanol | −0.0285 | 0.51 | 0.9982 | 100–5000 | 6 | B |
| Octanol | 0.00224 | 15.70 | 1.0000 | 0.5–54 | 7 | B |
| *trans*-2-Hexenol | −0.0006 | 1.52 | 0.9990 | 5–280 | 6 | B |
| Hexanol | −0.0649 | 3.21 | 0.9999 | 460–9000 | 5 | B |
| *Terpenes* | | | | | | |
| α-Terpineol | −0.0215 | 6.46 | 0.9984 | 2–145 | 6 | B |
| β-Citronelol | 0.00284 | 8.19 | 1.0000 | 1.3–130 | 8 | B |
| Nerol | 0.0106 | 7.14 | 0.9991 | 2.4–60 | 6 | B |
| Linalool | 0.004 | 15.60 | 1.0000 | 0.5–59 | 7 | B |
| Geraniol | −0.012 | 19.99 | 0.9974 | 1.2–60 | 7 | B |
| *Phenols* | | | | | | |
| 4-Ethylphenol | −0.0232 | 3.49 | 0.9997 | 4.6–116 | 5 | B |
| Eugenol | −0.0232 | 3.49 | 0.9998 | 4.6–116 | 5 | B |
| *m*-Cresol | −0.0122 | 7.21 | 0.9999 | 1.71–43 | 5 | A |
| Guaicol | 0.00107 | 9.22 | 0.9986 | 0.82–41 | 6 | A |
| *Ketones and aldehydes* | | | | | | |
| 2-Heptanone | −0.0104 | 12.20 | 0.9997 | 1.6–40 | 5 | A |
| Acetophenone | 0.00408 | 17.20 | 0.9997 | 0.83–42 | 6 | A |
| β-Ionone | −0.001 | 8.49 | 0.9997 | 0.7–42 | 6 | B |
| Benzaldehyde | 0.122 | 9.14 | 0.9995 | 2.7–134 | 6 | A |
| α-Ionone | 0.0005 | 8.72 | 0.9994 | 0.82–41 | 6 | B |
| Hexanal | 0.071 | 14.20 | 0.9991 | 2.59–129 | 5 | A |
| *Miscellaneous* | | | | | | |
| Acetal | 0.0035 | 8.87 | 0.9985 | 1.64–41 | 5 | A |
| Acetoine | −0.0083 | 1.53 | 0.9980 | 4.7–128 | 6 | B |
| γ-Nonalactone | −0.0107 | 4.67 | 0.9996 | 2–124 | 6 | B |
| Methionol | 0.0145 | 0.96 | 0.9999 | 2.56–128 | 6 | B |

Fraction A = dichloromethane; fraction B = diethyl ether; $n$ = number of calibration points.

Table 4
Recoveries of wine volatiles added to three different wine samples (list of chemicals and purity)

| Compound | Purchased from | Purity | Added (μg/l) | Recovery (%) | | |
|---|---|---|---|---|---|---|
| | | | | Wine 1 | Wine 2 | Wine 3 |
| *Esters* | | | | | | |
| Amyl acetate | Chemservice | 99.5 | 3 | 103.2 | 100.4 | 98.4 |
| Diethyl malonate | Aldrich | 99 | 456 | 101.5 | 96.9 | 100.7 |
| Diethyl succinate | Aldrich | 99 | 2276 | o.r | o.r. | 96.8 |
| Ethyl cinnamate | Aldrich | 98 | 6 | 94.9 | 96.2 | 98.4 |
| Ethyl decanoate | Polyscience | 99.5 | 356 | 103.4 | 97.5 | 98.0 |
| Ethyl heptanoate | Polyscience | 99.5 | 14 | 107.0 | 100.0 | 103.5 |
| Ethyl hexanoate | Polyscience | 99.5 | 797 | 96.5 | 100.5 | 96.1 |
| Ethyl lactate | Aldrich | 98 | 2268 | 105.2 | 98.4 | 102.7 |
| Ethyl laurate | Polyscience | 99.5 | 19 | 101.6 | 97.4 | 94.3 |
| Ethyl octanoate | Polyscience | 99.5 | 495 | 97.0 | 95.4 | 97.2 |
| Ethyl 3-hydroxybutyrate | Aldrich | 99 | 5 | 92.3 | 114.5 | 101.8 |
| Benzyl acetate | Chemservice | 99.5 | 2 | 116.2 | 90.8 | 91.9 |
| Hexyl acetate | Chemservice | 98.5 | 38 | 97.9 | 93.9 | 97.1 |
| Isoamyl acetate | Chemservice | 99 | 1139 | 103.9 | 98.0 | 97.3 |
| Isobutyl acetate | Chemservice | 99.5 | 5 | 100.7 | 98.7 | 101.1 |
| Phenylethyl acetate | Chemservice | 98.5 | 5 | 97.4 | 100.0 | 103.5 |
| *Alcohols* | | | | | | |
| 2-Butoxyethanol | Chemservice | 97 | 2 | 92.4 | 97.1 | 92.4 |
| 2-Ethylhexanol | Chemservice | 99 | 5 | 101.8 | 96.9 | 100.4 |
| 2-Heptanol | Polyscience | 98 | 5 | 99.0 | 99.2 | 96.2 |
| Benzyl alcohol | Aldrich | 99 | 8 | 89.1 | 102.0 | 104.3 |
| Butanol | Merck | 99.5 | 23 | 104.7 | 107.3 | 103.8 |
| *cis*-3-Hexenol | Aldrich | 98 | 21 | 101.4 | 102.4 | 98.6 |
| Decanol | Aldrich | 99 | 2 | 103.7 | 99.49 | 5.7 |
| Heptanol | Polyscience | 98 | 3 | 101.6 | 98.4 | 102.2 |
| Octanol | Polyscience | 98 | 2 | 102.8 | 95.8 | 100.0 |
| *trans*-2-Hexenol | Aldrich | 96 | 11 | 107.1 | 95.5 | 100.9 |
| Hexanol | Sigma | 98 | 1875 | 100.3 | 98.2 | 100.9 |
| *Terpenes* | | | | | | |
| α-Terpineol | Chemservice | 99 | 6 | 96.2 | 97.2 | 97.1 |
| β-Citronelol | Aldrich | 98 | 5 | 95.4 | 100.4 | 97.3 |
| Nerol | Sigma | 98 | 2 | 101.2 | 99.6 | 102.9 |
| Linalool | Aldrich | 97 | 2 | 105.0 | 109.7 | 98.3 |
| Geraniol | Fluka | 99.5 | 2 | 96.3 | 104.6 | 106.3 |
| *Phenols* | | | | | | |
| 4-Ethylphenol | Aldrich | 99 | 28 | 91.9 | 91.2 | 96.1 |
| Eugenol | Aldrich | 99 | 5 | 89.9 | 99.4 | 106.0 |
| *m*-Cresol | Aldrich | 99 | 2 | 108.2 | 108.2 | 97.1 |
| Guaicol | Aldrich | 98 | 2 | 105.5 | 101.8 | 104.3 |
| *Ketones and aldehydes* | | | | | | |
| 2-Heptanone | Polyscience | 99.5 | 2 | 98.7 | 101.9 | 96.9 |
| Acetophenone | Aldrich | 99 | 2 | 103.6 | 101.8 | 100.0 |
| β-Ionone | Sigma | 98 | 2 | 102.6 | 94.8 | 102.6 |
| Benzaldehyde | Aldrich | 99 | 5 | 95.3 | 97.8 | 96.5 |
| α-Ionone | Sigma | 97 | 2 | 105.5 | 103.0 | 97.6 |
| Hexanal | Polyscience | 99.5 | 5 | 101.4 | 99.4 | 101.5 |
| *Miscellaneous* | | | | | | |
| Acetal | Chemservice | 99 | 2 | 105.5 | 98.8 | 100.0 |
| Acetoine | Polyscience | 99 | 5 | 73.6 | 98.3 | 101.7 |
| γ-Nonalactone | Aldrich | 97 | 5 | 100.2 | 87.9 | 103.4 |
| Methionol | Aldrich | 98 | 5 | 110.0 | 121.3 | 105.9 |

data are presented in Table 2. Repeatability is better than 5% R.S.D. for approximately 60 compounds, and better than 10% R.S.D. for 70. These combined data demonstrate that the proposed method is robust and is resistant to any environmental or batch-to-batch variations. Furthermore, these results are significantly better than those obtained with classical techniques. This is probably due to: (1) the elimination of the variability normally associated with evaporation processes [7], (2) the high degree of accuracy of cold on-column injections, and, (3) improvements in chromatographic peak integration due to separation of the wine extracts into two less complicated fractions prior to gas chromatographic separation.

The linearity of the method was studied by the production of synthetic wine samples containing varying amounts of wine volatiles (see methods). Calibration graphs were prepared for 49 different volatile compounds and, in most cases, the correlation coefficient (*r*) was better than 0.999. These data are shown in Table 3. Generally speaking, there were no important deviations from linear behaviour over two orders of magnitude. Detection limits were not accurately determined, but an inspection of Table 3 suggests that they are likely to be lower than 1 $\mu$g/l for the best extracted compounds (i.e. the less water soluble ones).

Finally, the recovery of the volatile compounds from the wine matrix was checked by analysing three different wine samples that had been spiked with known amounts of volatiles. These results are presented in Table 4. Recoveries for all volatile compounds were between 73.6 and 121.3% with a mean recovery of 99.9%.

## 6. Conclusions

The combination of micro-extraction, followed by selective sample clean-up by a silica solid-phase cartridge, provides an effective method for the analysis of wine volatiles. This approach has a number of advantages over classical techniques: (1) The use of micro-extraction eliminates the need of long and tedious evaporation steps. This improves the precision of measurements and helps to reduce the amount of solvents used. (2) The use of a silica cartridge to separate the wine extract into two fractions helps to prevent major volatile compounds from overlapping minor constituents in the gas chromatogram. This inevitably improves the precision of the chromatographic measurement.

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